S/N 10/684,129 **PATENT**

Applicant: Stacey L. Schultz-Cherry et al. Examiner: Benjamin P Blumel
Serial No.: 10/684,129 Group Art Unit: 1648
Filed: October 10, 2003 Docket No.: 800.039US1
Title: METHOD TO DETECT ANTIBODIES SPECIFIC FOR TYPE-2 TURKEY ASTROVIRUS

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

We, Dr. Stacey Schultz-Cherry and Dr. Matthew Koci, declare and say as follows:

1. We are the named co-inventors of subject matter claimed in the above-identified application, Serial No. 10/684,129, filed on October 10, 2003, and make this Declaration in support of the patentability of the claims in the above-identified application.

2. In the Office Action dated January 25, 2007, the Examiner rejected claims 1-7, 12-23, 27-32, and 34 under 35 U.S.C. § 103(a) as being unpatentable over Schultz-Cherry et al. (U.S. Patent No. 6,696,562), Gulati et al. (J. Clin. Microbiol., 38:4010 (2000)), Black et al. (J. Gen. Virol., 74:143 (1993)), Koci et al. (J. Virol., 74: 6173 (2000)) and Kim et al. (WO 92/20803).

3. The Examiner is of the opinion that it would have been obvious to one of ordinary skill in the art to modify the method taught by Schultz-Cherry et al. in order to detect antibodies specific for turkey astrovirus-2 capsid antigen, that one would have been motivated to do so, given the suggestion by Schultz-Cherry et al. to screen for immunoglobulins in biological fluids of an infected subject, and that there would have been a reasonable expectation of success, given Gulati et al., Black et al., Koci et al., and WO 92/20803.

4. Although Gulati et al. and Black et al. disclose serological assays for avian pneumovirus and influenza virus, respectively, and the '562 patent and WO 92/20803 generally disclose methods of using anti-astrovirus antigens, none of the cited documents teaches or suggests an assay which actually detects anti-astrovirus antibodies in a blood sample. As

discussed in a Rule 132 Declaration filed on November 30, 2006 in the above-referenced application, there are a number of obstacles to overcome when designing a serological assay.

5. After turkey astrovirus-2 was isolated, individuals from the poultry industry approached us to develop a serological assay to detect turkey astrovirus-2 infection, a virus associated with poultry enteritis and mortality syndrome (PEMS).

6. Before an agent was identified that was associated with PEMS, we used phage display technology to characterize pathogens associated with PEMS. Turkey astrovirus-2 proteins (not limited to capsid proteins) were thus part of the phage library. Those displayed proteins were exposed to sera from infected animals. No reactivity was observed.

7. Various expression systems for turkey astrovirus-2 capsid were tested in order to obtain immunogen for hyperimmune sera production. Those systems included *in vitro* transcription/translation, transient expression in mammalian cell lines, and expression in bacterial cells. Yields were generally poor and/or variable. Of the tested systems, the *in vitro* transcription/translation system produced the best results and was initially chosen for developing the serological assay. Issues related to consistent antigen preparation led to concerns in the reproducibility of ELISA and ELISPOT assays that used capsid produced by *in vitro* transcription/translation to detect anti-turkey astrovirus-2 antibodies in serum.

8. To obtain positive sera from naïve birds, turkeys were inoculated with plasmid encoding turkey astrovirus-2 capsid, inactivated virus, live virus or capsid protein produced by *in vitro* transcription/translation. Only low amounts of virus-specific antibodies were obtained using the tested immunogens. Similar results were seen in inoculated chickens and mice.

9. Based on the observations described above, it is likely that the amount of anti-turkey astrovirus-2 antibodies produced in animals exposed to turkey astrovirus-2 antigen, for example, in infected birds, is less than antibodies to other viruses or antigens (see Koci et al., J. Virol., 78:1564 (2004)). Alternatively or additionally, those antibodies may not have a high

binding affinity. Therefore, any assay useful to detect anti-turkey astrovirus-2 antibodies must result in sufficient specific binding and low non-specific binding.

10. Of the antigens tested, only turkey astrovirus-2 capsid expressed in insect cells reproducibly and specifically detected anti-turkey astrovirus-2 antibodies in serum. There is nothing in the cited art that teaches or suggests the claimed assay or provides a reasonable expectation that a particular assay would be useful to detect anti-turkey astrovirus-2 antibodies in serum.

11. We further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: ________________ By: ______________________________
Dr. Stacey Schultz-Cherry

Dated: 5/24/2007 By: ______________________________
Dr. Matthew Koci